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No. .....

Copenhagen S., 28 March 19 56.
(Denmark)

Professor Joshua Lederberg
Department of Genetics
College of Agriculture
The University of Wisconsin
Madison 6, Wisconsin
U.S.A.

Dear Dr. Lederberg,

Thank you very much for your kind letter regarding the views I presented in my note on antibody formation. I now enclose a reprint of this paper.

Since you mention that you have been told that there is a certain degree of similarity between Ehrlich's own views and my hypothesis, I enclose a reply I have just sent to professor Haurowitz who has pointed out to me both this similarity and his disbelief in the preformed existence of antibodies against "strange products of the chemical laboratory".

I was very happy to have your letter, and as you say "at least one second for my proposal". No others have come forth since I published these ideas but I am more content to have you than the whole clan of immunologists.

Yours sincerely

Niels K. Jerne

N.K. Jerne

NKJ/AT 28 March 56

Dr. Felix Haurowitz,
Professor of Chemistry,
Indiana University
Bloomington, Indiana

Dear Dr. Haurowitz,

Thank you very much for your letter concerning my paper on the Natural-Selection Theory of Antibody Formation.

I am, indeed, sorry now that I did not mention Paul Ehrlich in my paper, since the similarity between his famous theory and mine has been pointed out to me by you and also by other readers. However, I did not consciously derive my ideas on antibody formation from Ehrlich, and as my manuscript for the Proceedings of the National Academy of Sciences had to be short I could not include a historic account of antibody formation theories. Current textbooks on immunology describe Ehrlich's theory as "obsolete" and "of historic interest only". In my paper I presented an absolutely minimal statement of the two only theories which seemed to be seriously considered at present, namely yours and Burnet's. Moreover, it had never struck me that there was a close conceptual similarity between Ehrlich's theory and mine, and I cannot even now see that this be so. However, others may feel that I am mistaken, and in that case I shall be content to be considered somebody who tried to revive the interest in Ehrlich's ideas.

It is true that Ehrlich assumed, as I have also done, the preformed existence of all types of antibodies, or "receptors" as he said. But is this a sufficient reason to call his theory "very similar" to mine? Theories could be divided into two groups according to whether they assumed the preformed existence of antibody, or assumed the induction of an antibody structure de novo by the antigen. This does not mean, however, that the theories within each group must necessarily be very similar.

Ehrlich first proposed his theory in 1897, in Klinisches Jahrbuch, 6, pp. 299-326, Die Wertbemessung des Diphtherieheilserums, B. Ueber die Antitoxinwirkung. Theorie der Immunität. He elaborated on these ideas during the following years, and gave a clear exposition in his Croonian Lecture, read on March 22, 1900, before the Royal Society in London (Proc. of the Royal Society of London, 66, pp. 424-428, 1900). Ehrlich first assumes that toxin (antigen) possesses a haptophore atomic group which fits to a corresponding toxophile atomic group on the antitoxin. This toxophile group on the antibody molecule preexisted in a cell, as part of a nutritive side-chain. I had better cite Ehrlich directly from his above mentioned English publication pp. 432-436:

> We now come to the important question of the significance of the toxophile groups in organs. That these are in function specially designed to seize on toxines cannot be for one moment entertained. It would not be reasonable to suppose that there were present in the organism many hundreds of atomic groups destined to unite with toxines, when the latter appeared, but in function really playing no part in the processes of normal life, and only arbitrarily brought into relation with them by the will of the investigator. It would indeed be highly superfluous, for example, for all our native animals to possess in their tissue atomic groups deliberately adapted to unite with abrin, ricin, and crotin, sub-

stances coming from the far distant tropics.

One may therefore rightly assume that these toxophile protoplasmic groups in reality serve normal functions in the animal organism, and that they only incidentally and by pure chance possess the capacity to anchor themselves to this or that toxine.

.....

We may regard the cell quite apart from its familiar morphological aspects, and contemplate its constitution from the purely chemical standpoint. We are obliged to adopt the view, that the protoplasm is equipped with certain atomic groups, whose function especially consists in fixing to themselves certain food-stuffs, of importance to the cell-life. Adopting the nomenclature of organic chemistry, these groups may be designated side-chains. We may assume that the protoplasm consists of a special executive centre (Leistungs-centrum) in connection with which are nutritive side-chains, which possess a certain degree of independence, and which may differ from one another according to the requirements of the different cells. And as these side-chains have the office of attaching to themselves certain food-stuffs, we must also assume an atom-grouping in these food-stuffs themselves, every group uniting with a corresponding combining group of a side-chain. The relationship of the corresponding groups, i.e. those of the food-stuff, and those of the cell, must be specific. They must be adapted to one another, as e.g. male and female screw (Pasteur), or as lock and key (E. Fischer). From this point of view, we must contemplate the relation of the toxine to the cell.

We have already shown that the toxines possess for the antitoxines an attaching haptophore group, which accords entirely in its nature with the conditions we have ascribed to the relation existing between the food-stuffs and the cell side-chains. And the relation between toxine and cell ceases to be shrouded in mystery if we adopt the view that the haptophore groups of the toxines are molecular groups, fitted to unite not only with the antitoxines but also with the side-chains of the cells, and that it is by their agency that the toxine becomes anchored to the cell.

We do not, however, require to suppose that the side-chains, which fit with the haptophore groups of the toxines, i.e., the side-chains which are toxophile, represent something having no function in the normal cell economy. On the contrary, there is sufficient evidence that the toxophile side-chains are the same as those which have to do with the taking up of the food-stuffs by the protoplasm. The toxines are, in opposition to other poisons, of highly complex structure standing in their origin and chemical constitution in very close relationship to the proteids and their nearest derivatives. It is, therefore, not surprising if they possess a haptophore group corresponding to that of a food-stuff.

.....

The theory above developed allows of an easy and natural explanation of the origin of antitoxines. In keeping with what has already been said, the first stage in the toxic action must be regarded as being the union of the toxine by means of its haptophore group to certain "side-chains" of the cell protoplasm. This union is, as animal experiments with a great number of toxines show, a firm and enduring one. The side-chain involved, so long as the union lasts, cannot exercise its normal physiological nutritive function - the taking up of definite food-stuffs. It is as it were shut out from participating, in the physiological sense, in the life of the cell. We are therefore now concerned with a defect which, according to the principles so ably worked out by Prof. Carl Weigert, is repaired by regeneration. These principles, in fact, constitute, the leading conception in my theory. If, after union has taken place, new quantities of toxine are administered at suitable intervals and in suitable quantities, the side-chains, which have been reproduced by the regenerative process, are taken up anew into union with the toxine, and so again the process of regeneration gives rise to the formation of fresh side-chains. In the course of the progress of typical systematic immunisation. as this is practised in the

> case of diphtheria and tetanus toxine especially, the cells become, so to say, educated or trained to reproduce the necessary side-chains in ever-increasing quantity. As Weigert has confirmed by many examples, this, however, does not take place as a simple replacement of the defect; indeed, over-compensation is the rule. Thus the lasting and ever-increasing regeneration must finally reach a stage at which such an excess of side-chains is produced that, to use a trivial expression, the side-chains are present in too great a quantity for the cell to carry, and are, after the manner of a secretion, handed over as needless ballast to the blood.
> .....

From the exposition of Ehrlich's, cited above, I would emphasize the following points:

1) Antibodies are pushed-off protoplasmic side-chains whose normal function in the cells was the grabbing of food.

2) Antigens are capable of inducing side-chain production (followed by push-off), because they have an atomic group in common with a type of food-molecules which some cells are equipped to accept.

3) Production of side-chains is due to the repair of a "defect". This defect consists in the lack of this type of side-chains because those previously available are now occupied by a firm union to antigen. Over-compensation of repair leads to excess production, and to "pushing off".

Contrary to these points, my natural-selection theory 1) does not assume that that antibodies have some other "normal" function in the cells that produce them, 2) does not assume that antigens necessarily have specific atomic groups in common with nutritive molecules, 3) does not see the stimulus to antibody production in the negative deprivation by elimination of a certain type, but in the positive selection of a certain class of globulines for reproduction.

This brings me to the next point in your letter, stating that "the great difficulty of Ehrlich's theory of preformed antibodies is to explain the formation of antibodies against such strange products of the chemical laboratories as for instance p-, m- and o- aminophenylarsonic acid or sulfanilic, metanilic and other peculiar acids". This is so, because Ehrlich wanted the preformed "receptors" to be adapted to the normal function of grabbing food for the cell, and it is hard to imagine cells equipped with specific food-grabbing protoplasmic "tentacles" in such a multiplicity as to cover all sorts of strange chemical groups. But the argument does not embarrass my theory.

I can perhaps make this clear by answering your final question "whether I really believe that in a rabbit there are globulins adapted to the p-azophenylarsonic acid group, others adapted to the m-azophenylarsonic acid group, and again others adapted to the m-azophenylsulfonic acid group".

The word "adapted" seems to me to imply that the cells which produce such globulins, or the ancestors of such cells, have had previous experience of the haptenic groups you mention. This is exactly what Ehrlich said: that the haptenic groups on antigens (or haptophore groups as he called them) were identical to atomic groups on "food" molecules of which the protoplasma of some class of cells had previous experience. My point is that there may very well be globulin molecules present in the blood of a rabbit which "fit" the haptenic group you mention, without being "adapted" to them.

If a printer wanted to print the word IVAR he might pick out the four letters, one by one, from a box containing many copies of each of 25 letters. Instead, however, he might have available a large collection of random combinations of letters and pick out a preformed IVAR combination. There are 25^4 or about 400,000 possible combinations of four letters, so if the collection of random combinations contained 10^{17} specimens (the number of globulin molecules in 1 ml of serum), there would very likely be present more than 10^{11} individuals showing the combination IVAR. If the correct combinations were to be picked out by an antigen device working on the basis of some sort of "affinity", we could easily imagine that related combinations might be picked out also, such as IVOR, IVAN, LIVAR, etc.

Reproduction of the class of structures selected would thus lead to both "specificity", and to "cross-reactions" with similar antigens.

The argument that we cannot imagine the preformed presence of globulin molecules "fitting" all sorts of "artificial" haptenic groups, contains, I think, the following underlying fallacies:
1) any atomic group we can synthesize can act as a haptenic group
2) the number of haptenic groups is infinite
3) the antigen-antibody relation is a strictly specific one-to-one relation.

(1) is not true, because the epithet "haptenic" is given only to certain atomic groups. When no or a poor antibody response is obtained we say that we are dealing with poor haptens or poor "determinants". Substitutions can be made into haptenic groups which do not markedly change their specificity.
(2) is a question of large numbers. Even if (what I think very unlikely) as many haptenic groups of different specificity could be synthesized as there are names in the New York telephone directory, this would amount to only about one million, whereas the number of globulin molecules in the blood of a rabbit is more than a million times a million times a million.
(3) Everybody has shown that the antibodies produced in response to an antigen are <u>not</u> strictly specific, not even those produced in response to a well-defined chemical group. This means that one haptenic group can lead to the production of a class of antibody molecules, each of which possesses a configuration which will fit more or less closely to members of a class of haptenic groups.

I therefore believe that it is very well possible that a rabbit contains, as the result of a more or less "random" synthesizing mechanism, globulin molecules which will fit any antigen to which this rabbit can respond, including the chemical substances you mention in your letter.

Finally, I should like to ask you: Do you really believe that the "strange products of the chemical laboratory" which you mention are admitted into the globulin assembly line of the workshop of an antibody producing cell and there can preside over the creation of thousands of complementary globulin molecules? This seems to me far more fantastic than the mechanism I have suggested. But, of course, our "Do you really believe" questions are merely rhetorical, since the value of ideas cannot be tested by the sincerity of the proponents.

I am more inclined to believe rumours I have heard: that Šterzl in Praha, Czechoslovakia, claims to have succeeded in obtaining antibody production in an animal after injection of nucleic acid, prepared from the lymphoid cells of an immunized animal.

Hoping to hear your comments, I remain, with kind regards

Yours sincerely

N.K. Jerne

P.S. I have sent a copy of this letter to professor Joshua Lederberg who has shown interest in my hypothesis.